Digestion

1.Enzyme A 1μl
Buffer（10X） 0.2μl
BSA(100X） 2μl
ddH_2O rest of the volume
incubate at recommended temperature (37℃) for at least 2 hour, and temperature（68℃）for 10 mins
2.Purify the digestion product;
3.Enzyme B 1μl
Buffer（10X） 0.2μl
BSA(100X） 2μl
ddH_2O rest of the volume
incubate at recommended temperature (37℃) for at least 2 hour, and temperature（68℃）for 10 mins
4.Purify the digestion product;

Notes:
The enzymes used here are NEB enzymes (EcoRI / XbaI / SpeI / PstI), and buffer4 is suitable for most of double digestion;
For 50 μl reaction system, the suggested amount of each restriction enzyme is 2μl;
According to personal experience, 50μl reaction system has lower efficiency than 20 μl reaction system, so 20μl reaction system is recommended.